Typhoid fever is an important public health concern in India. Human typhoid fever is a severe systemic infection of reticuloendothelial system caused by the bacterium Salmonella typhi. A few years back, the mean incidence of typhoid fever in developing countries has a ratio of 150/10⁶/year in South America to 1000/10⁶/year in Asian countries, the situation remains almost same even today (Ivanoff et al. 1994). Rats and mice are used as animal model for in vivo studies and in these animals salmonellosis is caused by S. typhimurium. Both S. typhi and S. typhimurium, the causative organisms for human and rodents/murines respectively, have been extensively used to understand the pathophysiology of disease (Hsu, 1989). Salmonellae, Gram-negative bacilli, are facultative intracellular bacteria and can survive during certain stages of host-parasite interactions (Mroozenskey-Wildeyc^1989). The organisms gain entry into the host by oral route following ingestion of contaminated food, water and vegetables. The onset of disease takes 5 to 20 days, depending on the health and immune status of the individual and the number of organisms ingested. Often the first sign of infection is enterocolitis with diarrhea, frequently accompanied by non-specific symptoms of headache, chills, anorexia, weakness, dizziness, muscle pain and abdominal cramping. The symptoms of untreated typhoid fever begin to resolve by the fourth week of infection, although relapse occurs in approximately 10% of individuals apparently recovering from the infection (Hornick et al., 1970). Intestinal complications can occur in later stage of untreated infection; these include bleeding and perforation that are the result of an inflammatory response in the Peyer's patches followed by necrosis and ulceration of the intestinal epithelium (Butler et al., 1985; Owen, 1994). Intestinal perforations usually result in peritonitis and often require surgical intervention. After its entry, the bacterium invades and multiplies in liver and spleen and then
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migrates to gall bladder and ultimately to intestine (Richter-Dahlfors et al., 1997).

To obtain a better understanding of the pathogenesis of typhoid fever, it seems crucial to elucidate the host responses against Salmonella with focus on defence oriented molecules such as oxygen radicals and nitric oxide (NO). Nitric oxide is certainly one of the smallest biological mediators (Nathan, 1992). NO is the smallest molecule (MW 30) of all the known bioactive mammalian cell secretory products. High chemical activity of NO results in its short half-life inside the cell and the specificity of its interaction is minimal. In mammalian cells NO is produced along with L-citrulline by the enzymatic oxidation of L-arginine. The antimicrobial role of NO has been well established. This activity of NO has been demonstrated by a variety of approaches. The most important is the NO production by inducible nitric oxide synthase (iNOS) which is stimulated with cytokines and microbial products such as lipopolysaccharide (LPS).

The production of NO by activated macrophages is the result of increased expression of inducible isoforms of iNOS that produces NO and citrulline from arginine. Once iNOS is expressed, the level of NO production is dependent upon the concentration of L-arginine, which is the only physiological nitrogen donor for NO production (Morris and Billair, 1994). Previous studies have shown that the physiological levels of L-arg are rate limiting for NO production (Lyenger et al., 1987; Nussler et al., 1994). L-cit, the by-product of NO synthesis can be recycled to arginine via arginosuccinate synthetase (AS) and arginosuccinate lyase (AL), thus, increasing the available L-arg for NO production in activated iNOS expressing cells (Nussler et al., 1994; Wu & Brosnan, 1992). The arginine biosynthesis enzyme and iNOS are co-induced upon stimulation of a macrophage cell line with interferon-gama (IFN-γ) and LPS. This co-induction correlates with increased enzymatic activity and NO production (Nussler et al., 1994). These results suggest that NO production and arginine biosynthesis are coupled, and that increased
extracellular arginine or citrulline could support increase in NO production in IFN-γ activated macrophage cell line (Morris & Billiar, 1994).

Objectives of the thesis

Cellular NO production is achieved by NOS activity, intracellular L-arg concentration, availability of various co-factors and cell responsiveness to specific cytokine and other stimulate. However, precise regulatory steps may vary in different cell types. Previous studies have suggested that exogenous administration of L-arg results in increased NO production, indicating that endogenous substrate is insufficient for maximal NO production. Taking these facts in consideration, it was thought pertinent to see the effect of oral administration of NO donors on NO production. We pretreated the mice with two NO donors (L-arg and L-cit), and also blocked the iNOS by a NO inhibitor compound (aminoguanidine) with or without infection with S. typhimurium and studied the bacteriocidal effect of these compounds against S. typhimurium infection and their impact on the host. Following were our specific objectives:

i) To study the role of NO donors in eliciting cellular protection against S. typhimurium (wild) infection in mice resulting in increased degree of survival.

ii) To study the biochemical mechanism(s) of action of NO donors particularly in relation to oxidative stress.

iii) To elucidate the immunomodulatory mechanisms involving NO donor compounds.